Version
2.1.2.0

Abstract

Grant Number: 1 X01MH78957-01
PI Name: Auld, Douglas S
PI Email: dauld@mail.nih.gov
Project Title: Identification of lkappaBalpha Stabilizers in a Human Lymphoma Cell Line Using A

Abstract: *DESCRIPTION (provided by applicant):* The goal of this project is to identify chemical probes that inhibit "upstream" points in activation of the NF-kappaB (NFkB) pathway using cell lines from a type of lymphoma that is largely incurable by conventional chemotherapy. Many cell types normally activate the NFkB signaling pathway temporarily in vital functions such as immunity, but certain cancers display constitutive NF-kB activation and can resist therapy. While it is clear that the later stages of NF-kB activation are common to both normal and malignant cells, the upstream mechanisms of constitutive NF-kB activation in cancer are largely unknown. In the classical pathway of NF-kB activation, the midpoint is rapid degradation of the critical regulatory protein IkappaBalpha (IkBa) after phosphorylation by an activated IkappaB kinase (IKK) complex. We have found that cell lines of the ABC-DLBCL type of human lymphoma display high constitutive IKK activity, on which they depend for survival. Conventional cell-based assays of NF-kappaB activation measure its endpoint, and present several problems for HTS. We have addressed these with an innovative cell-based assay in ABC-DLBCL lines that interrogates the midpoint of the NF-kB pathway. In stably-engineered reporter lines, green light-emitting beetle luciferase is fused to IkBa and increases upon inhibition of IkBa degradation,while unfused red luciferase provides for normalizing the signal to cell number and check cell viability. Development and testing of this assay has confirmed its precision, robust response to known small-molecule inhibitors of IKK activity or IkBa degradation, and suitability for miniaturization and HTS. Screening to date of 11,707 compounds has yielded a small number of actives, some of which are structurally similar to known IKK inhibitors. Further screening will increase the chance that compounds can be found that inhibit upstream causes of constitutive NFkB activation in these lines, or identify novel inhibitors of IKK activity or IkBa degradation, with the potential for mechanistic insights and therapy development. Large-scale validation of this assay will promote its use in other applications, such as cell lines from other types of cancer with constitutive NFkB activity. The chemical probes yielded by the project should be useful tools in providing a better understanding of this process and perhaps leading to therapies that would

specifically inhibit constitutive NFkB activation in cancers without causing side effects such as immunosuppression.

Thesaurus Terms: chemical probes, NF-kappaB, NFkB, lymphoma, chemotherapy, cancer, IkappaBalpha, IkBa, IkappaB kinase, IKK, ABC-DLBCL, cell-based assays, High-throughput screening, HTS, small molecule inhibitors, immunosuppression

Institution:	NIH CHEMICAL GENOMICS CENTER NATIONAL HUMAN GENOME RESEARCH INSTITUTE, NIH 9800 MEDICAL CENTER DR BUILDING C, RM 3201 ROCKVILLE, MD 208523370
Fiscal Year:	2007
Project Start:	2006/07/25
Project End:	2007/07/25
ICD:	NATIONAL INSTITUTE OF MENTAL HEALTH
IRG:	ZMH1